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Quantitative analysis of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in a kratom (*Mitragyna speciosa* Korth.) cocktail using high-performance liquid chromatography

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ABSTRACT

A simple HPLC technique for determining mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in 'kratom cocktail' was developed. The analytical method for mitragynine, codeine and caffeine used an Eclipse XDB-C8 column. A Lichrospher CN column was using for analysing chlorpheniramine and phenylephrine. The correlation coefficient of each standard was between 0.9957 and 0.9993. The precision of the methods were between 0.700 and 7.108% RSD. The concentration of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in 'kratom cocktail' was 90.021, 234.174, 73.986, 7.053 and 1.486 mg/L, respectively.

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1. Introduction

Kratom (*Mitragyna speciosa* Korth.) in the family Rubiaceae, is a tropical tree indigenous to Thailand, Malaysia, Myanmar and other areas of South East Asia. The tree reaches heights of 50 ft with a spread of over 15 ft. Extracts from leaves of this tree contain many alkaloids that have psychoactive effects. There are two main kinds of kratom distinguished by the color of their leaf veins – red or green. The red veined variety is supposed to have a stronger effect. Over 25 alkaloids have been isolated from kratom, of which the indole alkaloid, mitragynine, is the most important. Mitragynine, chemically known as 9-methoxy-corynantheidine (Fig. 1), is the primary active alkaloid in the plant.

Kratom has been used by natives of Thailand and other regions of Southeast Asia as a herbal drug for decades and perhaps forever. Traditionally, kratom was mostly used as a stimulant by Thai peasants, laborers, and farmers to overcome the burdens of hard work. Thai natives chewed the leaves to make them work harder and feel good. Kratom was also used to alleviate opium withdrawal. A kratom user mostly likes to chew fresh leaves as the compounds in the leaf veins are then more easily extracted. Dried leaves can also be chewed, but since they are a bit tough, most people prefer to crush them up or powder them so that they can be swallowed. Heavy users may chew kratom between 3 and 10 times a day. While new users may only need a few leaves to obtain the desired effects, some users find that with time they need to increase the dose to 10–30 leaves or even more per day. Kratom has been reported to be a central nervous system stimulant, and also a depressant. It helps to increase work efficiency and tolerance to hard work under a scorching sun [1]. Kratom is also used to treat muscle ache and fatigue because it can produce muscle relaxation [2]. It can be a very effective treatment for diarrhea [3]. In 1979, the Thai government enacted the Narcotics Act B.E. 2522, placing kratom along with marijuana in Category V of a five category classification of narcotics. This means that it is illegal to buy, sell, import, or possess. This law makes planting the tree illegal and requires existing trees to be cut down. This law however is not fully effective, since the tree is indigenous to the country and native people have always used them. Hence, kratom remains a popular drug in Thailand, especially in the Southern regions.

From the national household survey in 2007, kratom is the most popular drug within southern Thailand, the second and third is marijuana and methamphetamine, respectively [4]. The pattern of

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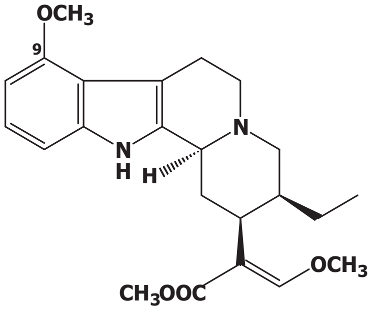

Fig. 1. Chemical structure of mitragynine.

use has more recently changed from chewing the fresh leaves to drinking a 'kratom cocktail' especially by teenager users. They often have a meeting everyday to drink this cocktail. The kratom cocktail (dubbed 4 × 100; 4 times 100) is a mixture of boiled kratom leaves, coke, antitussis syrup, coffee or codeine etc. and is served with ice. The origin of the name is unclear, but it likely came from its four or more ingredients. The users felt that kratom leaves and caffeine in soft drinks stimulated the nervous system, causing an increase in alertness, while the cough syrup and tranquilisers caused drowsiness. The above combination made the drinker high. 'Kratom cocktail' quickly spread to Bangkok where it is now popular in the poorer suburbs. Recently hitting the streets was the all new 5 × 100 and then quickly after the 6 × 100. New ingredients have included yoghurt, coffee or alprazolam, etc. The type and quantity of each ingredient was dependent on the users. People found with kratom leaves and antitussis syrup are still arrested by the police [5]. These cocktails may have an impact on certain health problems in long term users.

The aim of this study was to develop an analytical HPLC method to detect and quantify the main active substances in the kratom cocktail such as mitragynine, caffeine, codeine, chlorpheniramine and phenylephrine.

2. Experimental

2.1. Chemicals

Methanol, acetonitrile and isopropanol were supplied by J.T. Baker, Malaysia. Chlorpheniramine maleate, codeine, caffeine and phenylephrine were purchased from Sigma–Aldrich, USA. Pure mitragynine from kratom leaves was obtained by the described extraction procedure [6]. The pure compound had been analysed by MS (Thermofinnigan MAT 95 XL mass spectrometer: EIMS with a direct insert probe) and 1H-NMR and 13C-NMR (Varian Unity Inova 500 NMR spectrometer) spectra. When the obtained spectral data were compared with the published assignments [7,8], it was identified as mitragynine.

2.2. Sample

A sample of a 'kratom cocktail' (4 × 100) was obtainen from Pattani province, southern Thailand. It was checked for its volume and its pH was checked by a pH meter (Metler Toledo, USA). It was concentrated by a vacuum freezer drier (Corrosion Resistant Freezer Drier, FTS System Inc., USA). The dry sample was weighed and kept at −20 °C until assayed. The dry sample of 'kratom cocktail' was reconstituted in the column mobile phase and filtered through a nylon membrane (0.2 μm) before analysis.

2.3. Chromatographic instruments and conditions

The HPLC system consisted of an Agilent model 1100 series Diode array detector (Agilent, USA). The system was controlled and data analyses were performed with ChemStation software. The analytical method for mitragymine, codeine and caffeine was optimized using an Eclipse XDB-C8 column (5 μm, 150 mm × 4.6 mm i.d.; Agilent, USA). The analytical method for chlorpheniramine and phenylephrine was optimized using a Lichrospher CN column (LC) (5 μm, 250 mm × 4.0 mm i.d.; Agilent, USA). All solvents for the mobile phase and sample were filtered through a 0.20 μm Millipore filter.

HPLC analysis for mitragynine was performed by isocratic elution with a flow rate of 0.5 mL/min. The temperature was controlled at 35 °C. The detector wavelength was set at 221 and 291 nm. The mobile phase was methanol:water (80:20, v/v). A volume of 10 μL of each prepared solution and samples were injected into the column. The chromatographic run time was 10 min.

Codeine and caffeine was analysed by isocratic elution with a flow rate of 1.0 mL/min. The separation was carried out at 25 °C. The detector wavelength was set at 215 nm. The mobile phase was 0.01 M KH_2PO_4:methanol:acetonitrile:isopropanol (74:8:9:9, v/v/v/v). The injection volume of each prepared solution and samples was 20 μL. The chromatographic run time was 5 min.

The analytical method developed for chlorpheniramine and phenylephrine used the LC system. The temperature was controlled at 35 °C. The detector wavelength was set at 215 nm for chlorpheniramine and 270 nm for phenylephrine. Separation was carried out at 35 °C. The mobile phase was 10 mM phosphate buffer at pH 3.0: acetonitrile (96:4, v/v), with a flow rate of 1.5 mL/min. A volume of 10 μL of each prepared solution and sample was injected into the column. The chromatographic run time was 10 min.

2.4. Method validation

Validation of the analytical methods was performed in accordance with the ICH Harmonized Tripartite Guideline Topic Q2B (ICH Steering Committee, 1996).

Standard solutions of mitragynine containing (5.000–100.000 mg/L) were prepared in the column mobile phase. Caffeine and codeine were mixed (0.010–200.000 mg/L) in their mobile phase. Chlorpheniramine and phenylephrine containing 5.000–200.000 mg/L were prepared in acetonitrile:water (20:80, v/v). The five concentrations of standard were subjected to regression analysis to calculate the calibration equation and correlation coefficients. Linearity was obtained for compounds between peak-area ratios and concentrations of 1–1500 mg/L mitragynine, 0.010–200.000 mg/L codeine and caffeine and 0.400–500.000 mg/L chlorpheniramine and phenylephrine. The limit of detection (LOD) was established at a signal-to-noise ratio (*S*/*N*) of 3. The limit of quantification (LOQ) was established at a signal-to-noise ratio (*S*/*N*) of 10. LOD and LOQ were experimentally verified by 6 injections of compounds at the LOD and LOQ concentrations.

2.5. Suitability of the method

The chromatographic parameters such as resolution, selectivity and peak symmetry were satisfactory for the compounds analysed. The standard solution of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine was prepared at a concentration of 10.000 mg/L and was checked by injecting nine replicates. The relative standard deviation (RSD) was calculated according to the formula: RSD (%) = (standard deviation/mean value) × 100. The level of acceptance was not more than 15% of the RSD value.

2.6. Precision

The precision of the method (within-day variations of replicate determinations) was checked by injecting nine replicates of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine at their LOQ level. The precision is expressed as %age RSD at the LOQ level.

2.7. Accuracy

Working standard solutions of mitragynine, codeine, caffeine was prepared to give final concentrations of 5.000, 10.000, 40.000, 60.000, 80.000, 100.000 mg/L. Standard working solutions of chlorpheniramine and phenylephrine were prepared at final concentrations of 10.000, 40.000, 80.000, 100.000, 150.000, 200.000, 250.000, 300.000 mg/L. Each standard was injected with nine replicates. From the respective area of the eluant, the concentrations of mitragynine were calculated using the detector responses. The accuracy was defined in terms of the %age deviation of the calculated concentrations from the actual concentrations. The percentage recovered was calculated using the expression: Recovery (%) = (response after extraction/response after direct injection) × 100.

2.8. Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using a working standard solution in the same chromatographic system and column on different days. Only small differences in area-ratios were obtained and there was a good constancy in the retention times observed after a 48 h time period. The responses obtained on different days indicated that this method was capable of producing results with a high precision on different days.

3. Results

The 3D spectra of standard mitragynine and mitragynine in the sample were detected by a diode array detector shown in Fig. 2. The parameters of standard mitragynine codeine, caffeine, chlorpheniramine and phenylephrine were ckecked in a similar

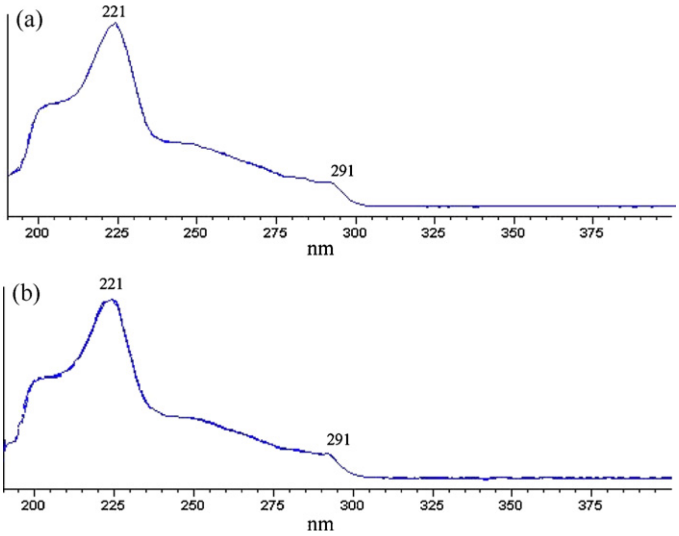

Fig. 2. The 3D spectra of standard mitragynine (A) and mitragynine in kratom cocktail (B).

way (Table 1). Linearities were evaluated by preparing six standard concentrations each of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine. The correlation coefficients of the standard active substances were all nearly 1.000. The LOQ values for mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine were 3.000, 0.010, 0.010, 0.400 and 0.400 mg/L and values for the LOD were 1.000, 0.005, 0.005, 0.200 and 0.200 mg/L, respectively (Table 2).

The retention times of standard mitragynine, codeine, caffeine, chlorpheniramine, phenylephrine were 5.969 ± 0.010, 1.939 ± 0.010, 2.340 ± 0.020, 3.159 ± 0.020 and 5.325 ± 0.030 min, respectively. The %age RSD for the precision, accuracy and ruggedness of each standard was less than 10% (Tables 3–5). The representative chromatogram of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine; and mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in 'kratom cocktail' is shown in Figs. 3–5.

The quantitative analysis for mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in the 'kratom cocktail' sample were 46.211, 41.222, 47.061, 1.711 and 0.252 g/kg, respectively (Table 6).

4. Discussion

Kratom has long been used by native people perhaps forever, especially in southern Thailand. The pattern of use has changed from chewing fresh leaves to drinking 'kratom cocktail'. The kratom cocktail is a mixture of boiled kratom leaves, coke, antitussis, coffee, etc. The composition and concentration of the recipe depends on the consumers. Kratom became illegal in Thailand but not illegal in European countries and USA. 'Krypton' is a herbal mixture contained kratom leaves and tramadol (a synthetic opioid analgesic), is quite popular in these countries [9]. Kratom leaves contain over 25 alkaloids including mitragynine, paynantheine, mitraphylline, and 7-hydroxymitragynine, etc. About 60% of the total alkaloids is mitragynine which is the main primary psychoactive constituent. The alkaloid patterns in young kratom leaves are quite difference from those in adult kratom leaves. Besides mitragynine, they also contain mitragynine

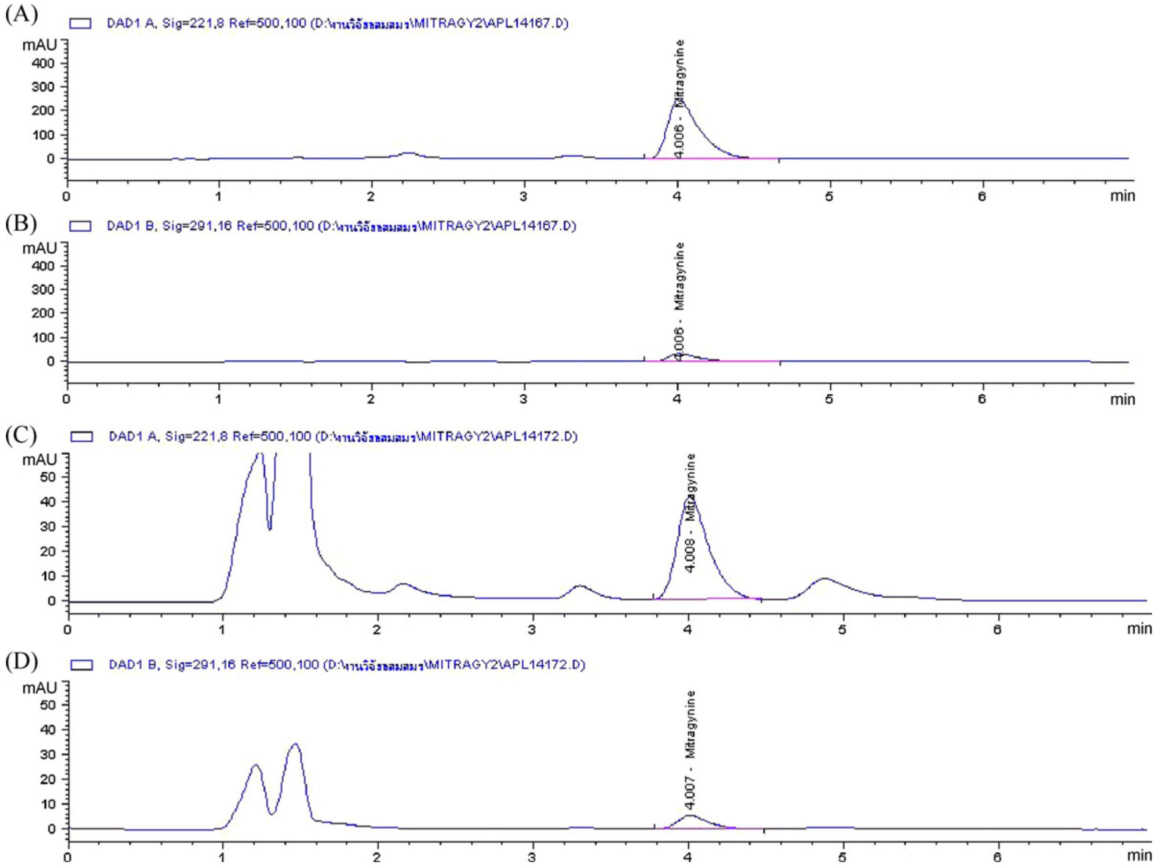

Fig. 3. Chromatogram of the standard mitragynine at 221 nm (A) and 291 nm (B), and mitragynine in kratom cocktail (4 × 100) at 221 nm (C) and 291 nm (D).

Table 1
System performance parameters of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine ($n = 9$).

Parameter	Mitragynine	Codeine	Caffeine	Chlorpheniramine	Phenylephrine	Preferable level
Injection precision	Mean: 324.665, %RSD 0.174	Mean: 688.869, %RSD 0.429	Mean: 1108.183, %RSD 0.559	Mean: 1086.452, %RSD 0.581	Mean: 226.567, %RSD 0.788	RSD < 1
Resolution	6.597	1.5	2.35	2.393	9.751	≥ 1.5
Symmetry	1.113	1.02	0.77	1.159	0.890	≤ 1.2
Selectivity	1.903	1.200	1.210	1.140	1.427	1.02-2.0
Theoretical plate	3224	2299	2851	3089	12068.57	≥ 2000

Table 2
Linearity, LOD and LOQ of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine.

Substance	Equation	r	LOQ (mg/L)	LOD (mg/L)
Mitragynine	$Y = 55.7738X + 213.6063$	0.9957	3.000	1.000
Codeine	$Y = 66.3072X + 37.3619$	0.9995	0.010	0.005
Caffeine	$Y = 107.7772X + 18.6298$	0.9997	0.010	0.005
Chlorpheniramine	$Y = 19.9957X - 21.1375$	0.9994	0.400	0.200
Phenylephrine	$Y = 17.8743X - 87.6477$	0.9993	0.400	0.200

Note: r = correlation coefficient; LOQ = limit of quantification; LOD = limit of detection.

diastereomer, the second abundant alkaloid [10]. This method was focused in mitragynine as the major psychoactive compound in kratom leaves. Mitragynine is the indication used by the officers of the Narcotics Control Broad of Thailand to detect the kratom leaf product and to prosecute the owners.

The active ingredients in a 'kratom cocktail' are normally mitragynine, codeine, caffeine, chlorpheniramine (antihistamine) or phenylephrine (decongestant) but the amounts vary depending on the users perference. These substances have effects on the central nervous system (stimulation or depression). Mitragynine is the major alkaloid in kratom leaves. Coke or Coca-Cola contains caffeine. The consumers may mix the drugs for treatment of the common cold in the kratom cocktail such as antitussis, antihistamine or decongestants. Qualitative and quantitative analyses for these active substances have been determined. Separation of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine was achieved here using a reverse phase liquid chromatography method. Mitragynine is insoluble in water but freely soluble in methanol. Hence, the proportions of methanol and water in the mobile phase determined the selectivity and sensitivity for separating mitragynine. In this method, a diode array detector could be used, not only for purity analysis of the peak, but also for specificity analysis. For peak purity analysis, a spectrum across the peak was compared with an average spectrum from the peak. If the data correlated well, a high purity factor was reported. If the data did not correlate well, then the peak was considered impure [11]. The result of the peak purities for mitragynine in the standard and sample were 998.871 and 997.045, respectively. The 3D spectra are

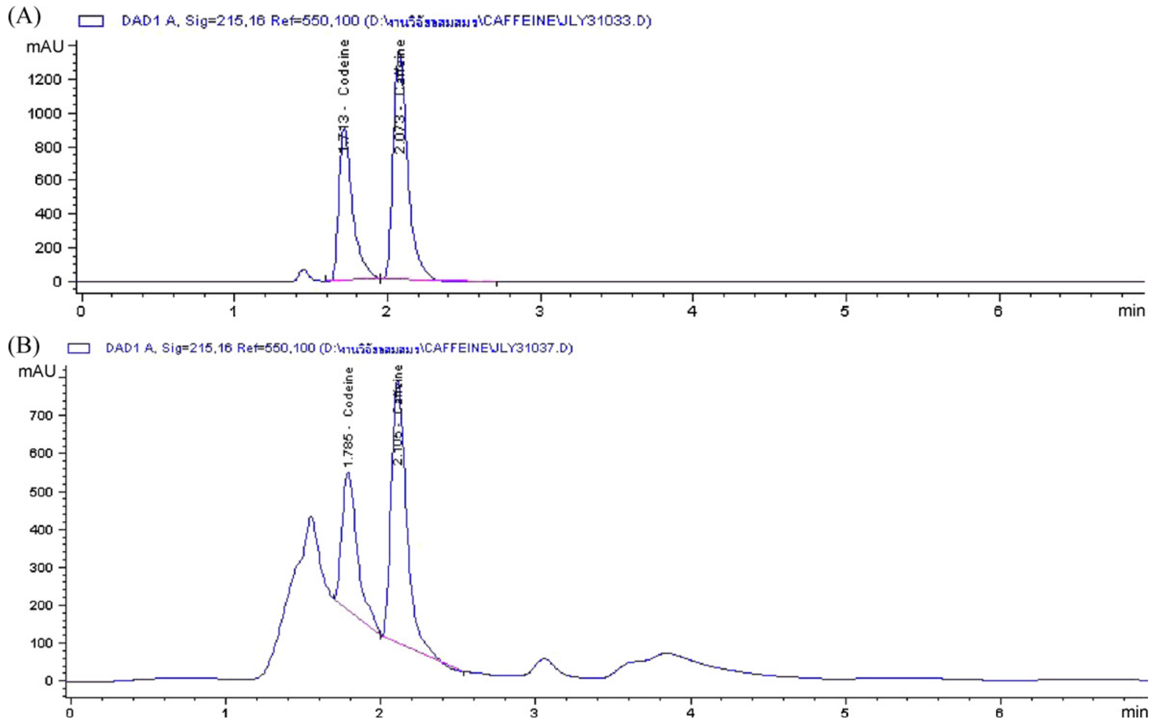

Fig. 4. Representative chromatogram of (a) standard codeine and caffeine; and (b) codeine and caffeine in kratom cocktail (4 × 100), retention time at 1.713 and 2.073 min; and 1.785 and 2.105 min, respectively.

Table 3
Precision of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine ($n = 9$).

Parameter	Mean± SD (mg/L)	%RSD
Mitragynine	2.820 ± 0.020	0.700
Codeine	0.219 ± 0.010	2.495
Caffeine	0.247 ± 0.000	1.123
Chlorpheniramine	1.024 ± 0.030	3.335
Phenylephrine	1.031 ± 0.070	7.108

Note: % RSD = % age relative standard deviation.

Table 4
Accuracy of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine ($n = 9$).

Substance	% RSD	% Recovery	% Deviation
Mitragynine	0.478	94.60	5.48
Codeine	0.834	98.96	1.04
Caffeine	0.220	95.83	4.17
Chlorpheniramine	0.243	100.56	0.56
Phenylephrine	0.840	97.02	2.98

Note: % RSD = % age relative standard deviation.

shown in Fig. 2. No deviations were seen in the beginning, at the apex and at the end of the peaks of standard mitragynine and sample. The specificity results were achieved from a simultaneous coupled wavelength data from the diode array detector (221 and 291 nm) as shown in Fig. 3. This clearly indicated that these compounds were pure and the proposed HPLC method had good specificity for measuring mitragynine.

The parameters used to determine the suitability of the system included the selectivity (α), resolution (R_s), plate number (N), peak symmetry (S) and injection repeatability (Table 1). All values fell within the acceptable values of the FDA guidelines [12]. The calculated value from the peak was analysed from the optimum HPLC condition while other parameters were matched the criteria. The HP ChemStation was not used to determine the asymmetry ratio of a peak, which is usually done, but by comparing the peak half-width at 10% of the peak height, or 5% as recommended by the FDA. Peak symmetry was calculated as follows: Peak symmetry (S) = B/A, this method has been previously described [11]. The result clearly indicated that the proposed HPLC method had good selectivity for measuring mitragynine and the other compounds.

For quantitative determinations, the linearities were evaluated by preparing six standard concentrations of each of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine. The correlation coefficients of the standard active substances were nearly 1.0 thus indicating the strength and direction of a good linear relationship. The LOQs and LODs were at very low concentrations (Table 2).

The retention times of standard mitragynine, codeine, caffeine, chlorpheniramine, phenylephrine were 5.969 ± 0.01, 1.939 ± 0.01, 2.340 ± 0.02, 3.159 ± 0.02 and 5.325 ± 0.03 min, respectively. The %age RSD for the precision, accuracy and ruggedness of each standard was less than 10% (Tables 3–5). These parameters were well within the level of acceptance. The representative chromatogram of standard mitragynine and mitragynine in 'kratom cocktail' was shown in Fig. 3. The method was very sensitive and accurate with a %age RSD of less than 5%.

The quantitative analysis of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in this 'kratom cocktail' by high performance liquid chromatography showed the presence of 90.021, 234.174, 73.986, 7.053 and 1.486 mg/L, respectively (Table 6). According to the isolation of mitragynine from kratom leaves, a dry crude alkaloid extract gave an approximate 0.25% yield based on the fresh leaf weight and contained 50% mitragynine [6]. Hence, the consumers will require fresh kratom leaves of more than 720 g in one litre of kratom cocktail. A very high concentration of codeine (234.174 mg/L) was mixed in this cocktail. Codeine is an opioid

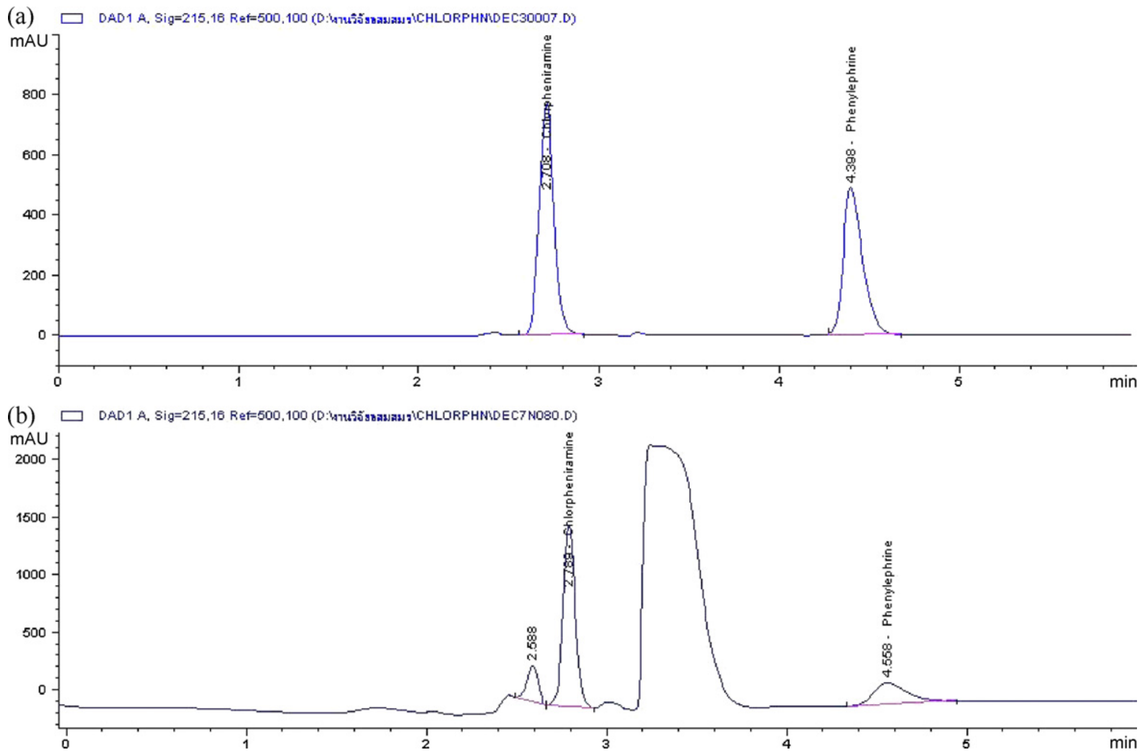

Fig. 5. Representative chromatogram of (a) standard chlorpheniramine and phenylephrine, and (b) chlorpheniramine and phenylephrine in kratom cocktail (4 × 100), retention time at 2.708 and 4.398 min; and 2.789 and 4.558 min, respectively.

Table 5
Ruggedness of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine for 3 days.

Substance	RT (min)		Peak area		Conc. (mg/L)	
	mean ± SD	%RSD	mean ± SD	%RSD	mean ± SD	%RSD
Mitragynine	7.829 ± 0.180	2.339	2032.444 ± 29.560	1.454	22.926 ± 0.000	0.011
Codeine	1.792 ± 0.010	0.776	2546.309 ± 78.711	3.091	43.145 ± 1.651	3.822
Caffeine	2.111 ± 0.022	0.807	4705.884 ± 63.850	1.357	49.580 ± 1.150	2.312
Chlorpheniramine	2.824 ± 0.010	0.344	2385.911 ± 3.241	0.136	105.143 ± 1.560	1.482
Phenylephrine	4.364 ± 0.032	0.641	672.293 ± 47.282	7.031	37.537 ± 0.481	1.280

Note: RT = retention time, AUC = area under the curve, Conc. = concentration, %RSD = %age relative standard deviation.

Table 6
Quantitative analysis of active substances in 'kratom cocktail'.

Substance	Concentration (mg/L)
Mitragynine	90.021
Codeine	234.174
Caffeine	73.986
Chlorpheniramine	7.053
Phenylephrine	1.486

agonist and it is classified as a CNS depressant. A small fraction (approximately 10%) of administered codeine is O-demethylated to morphine. Codeine and its salts (sulfate or phosphate) are administered to patients at doses of 15–60 mg, four to six times a day. The maximum daily dose for the relief of pain is 360 mg [13]. Caffeine present in the kratom cocktail was in the right concentration range for drinking. Since, Coca-Cola Classic contains 2.92 mg of caffeine per fluid ounce [14] and normal brewed coffee has 80–135 mg caffeine per serving [15]. The basis for the popularity of all caffeine-containing beverages is their stimulatory and antisoporific actions that elevates mood, decreases fatigue, and increases the capacity for work. The concentration of chlorpheniramine was in the therapeutic range (5–20 mg for a single dose). Chlorpheniramine is an H1 antagonist. Diminished alertness, slowed reaction times, and somnolence are common manifestations. Phenylephrine is a sympathomimetic drug (a selective α_1 agonist). Its concentration in the 'kratom cocktail' was very low. This concentration is not likely to have any significant effect on the human body.

The kratom cocktail users felt that it was a new herbal tonic. It comes down to keeping body and mind synchronized, to keep both of them fit under the complications of modern life, and especially to bring them back to form once they begin to feel fatigue, pain and depression. Since a kratom leaf is only a category V narcotic, the penalty is not severe. The offender shall be liable to imprisonment for a term not exceeding two years or to a fine not exceeding forty thousand baht or to both [16]. Police now use section 12 of the Medicines Act, which interprets the making of the cocktail as production of an unlicensed medicine, because it has cough syrup as an ingredient. Since codeine was classified as a category II narcotic, this offence carries a maximum five years in jail. This determination method will be useful for government officers who are concerned about the violation of the law and public health.

5. Conclusions

A reversed phase high performance liquid chromatographic method using a diode array detector for the determination of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine was developed. The chromatographic technique was simple, sensitive, precise and accurate for analysis of active compound in a kratom cocktail. The active substances detected were mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine. These substances have either a stimulatory or depressive effects on human subjects. The effect of a 'kratom cocktail' should be further investigated scientifically for its effect on human behavior and organ systems.

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References

[1] S. Suwanlert, A study of kratom eaters in Thailand, Bull. Narc. 27 (1975) 21.
[2] S. Chittrakarn, N. Keawpradub, K. Sawangjaroen, S. Kansenalak, B. Janchawee, The neuromuscular blockade produced by pure alkaloid mitragynine and methanol extract of kratom leaves (*Mitragyna speciosa* Korth.), J. Ethnopharmacol. 129 (2010) 344–349.
[3] S. Chittrakarn, K. Sawangjaroen, S. Prasettho, B. Janchawee, N. Keawpradub, Inhibitory effects of kratom leaf extract (*Mitragyna speciosa* Korth.) on the rat gastrointestinal tract, J. Ethnopharmacol. 116 (2008) 173–178.
[4] S. Assanangkornchai, A. Aramrattana, U. Perngparn, M. Kanato, N. Kanika, A. Ayudhya, Current situation of substance-related problems in Thailand, J. Psychiatr. Assoc. Thailand 53 (Suppl. 1) (2008) 24S–36S.
[5] Kratom, http://www.dailynews.co.th/newstartpage/index.cfm?page=Search (accessed 14.06.10).
[6] B. Janchawee, N. Keawpradub, S. Chittrakarn, S. Prasettho, P. Wararatananurak, K. Sawangjareon, A high-performance liquid chromatographic method for determination of mitragynine in serum and its application to a pharmacokinetic study in rats, Biomed. Chromatogr. 21 (2007) 176–183.
[7] P. Houghton, A. Latiff, I. Said, Alkaloids from *Mitragyna speciosa*, Phytochemistry 30 (1991) 347–350.
[8] E. Shellard, Ethnopharmacology of kratom and the *Mitragyna* alkaloids, J. Ethnopharmacol. 25 (1989) 123.
[9] T. Arndt, U. Claussen, B. Gussregen, S. Schrofel, B. Sturzer, A. Werle, G. Wolf, Kratom alkaloids and O-desmethyltramadol in urine of a Krypton herbal mixture consumer, Forensic Sci. Int. 208 (2011) 47–52.
[10] A. Philipp, D. Wissenbach, A. Weber, J. Zapp, H. Maurer, Metabolism studies of the kratom alkaloids mitraciliatine and isopaynantheine diastereomers of the main alkaloids mitragynine and paynantheine, in rat and human urine using liquid chromatography–linear ion trap-mass spectrometry, J. Chromatogr. B 879 (2011) 1049–1055.
[11] Hewlett-Packard, Understanding Your ChemStation, fifth ed., Hewlett-Packard, USA, 1998, p. 252.
[12] Reviewer Guidance Center for Drug Evaluation and Research (CDER), Validation of Chromatographic Methods. www.fda.gov/GuidanceCompliance Regulatory Information/Guidance.pdf, 1994 (accessed November, 1994).
[13] J. Reynolds, Martindale: The Extra Pharmacopoeia, The Pharmaceutical Press, London, 1993, pp. 1069–1071.
[14] Caffeine Content of Coca-Cola Classic. http://www.energyfiend.com/the-caffeine-database (accessed 12.09.11).
[15] Caffeine. http://en.wikipedia.org/wiki/Caffeine (accessed 12.09.11).
[16] Thailand Law Forum. http://www.thailawforum.com/database1/Narcotics-Act-part2-010.html (accessed 12.09.11).

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